Jour. agric. Res. China (中華農業研究) 29(3) : 183~194 (1980)

Calcium-45 mobility in young apple trees grown under different nutrient-calcium conditions[1]

Tsai Fua Chiu[2]

Abstract : Root-applied ^{45}Ca was readily translocated to leaves of normal-Ca trees but was less readily translocated to leaves of high-K or low-Ca trees. Calcium-45 accumulated to a much greater extent in roots and stems of the latter than in the former trees. Root-applied ^{45}Ca was translocated much more readily to apical young leaves than to basal old leaves of growing shoots. Shoot-applied ^{45}Ca was rapidly translocated to all leaves of excised shoots through the xylem transpiration stream. Calcium-45 applied to trees at the later stages of fruit development scarcely entered the fruit but accumulated strongly in the leaves of the fruit truss, and especially in the attachment point of the fruit (future abscission zone). The general nutritional status of trees changes the mobility of Ca through its effect on Ca-fixing sites in the plant tissues. A hypothesis of continuously-developing calcium-fixing sites in growing plant tissues has been proposed.

The translocation and distribution of Ca in apple trees has received much attention, due largely to the occurrence and economic importance of physiological disorders of the fruit associated with Ca deficiency. Calcium uptake, mobility and redistribution in plants is influenced by other nutrients and by internal regulatory systems.

Lewis and Martin [7] reported that, after branch injection of ^{45}Ca-labelled solution, radio-activity in mature apple fruits was found mostly in the stem end ; leaves and buds accounted for 95% of recovered activity, and the fruit for only 5%. Shear and Faust [11] stated that Ca translocation, in the stems of apple trees, is affected by means of a nonspecific ion exchange mechanism. Any divalent cation, in sufficient amount, can free adsorbed Ca for transport. The exchange sites may be a property of lignin. Millikan and Hanger [8] demonstrated that movement of previously deposited ^{45}Ca in *Trifolium subterraneum* L. (subterranean clover) could be induced by foliar injection of various non-radioactive cations. Jansen et al. [5] indicated that pectic materials present in cell walls of the coleoptile tissue could easily account for the total exchange capacity of

1. Contribution No. 935 from Taiwan Agricultural Research Institute. The author wish to express his sincere thanks to Dr. C. Bould, Long Ashton Research Station, University of Bristol, U. K., who assisted this work and corrected the manuscript.

2. Senior Specialist, Deptartment of Agricultural. Chemistry. TARI, Wufeng, Taichung Hsien, Taiwan 431, ROC.

that tissue. Pitman et al. [10] reported that a large part of the Ca^{++} exchange between cell and medium is associated with the fixed negative charges in the Donnan free space, located in the cell wall.

However, the precise nature of the Ca fixing sites in plant tissues and how they affect Ca mobility in plants is still not clear. These experiments, conducted in sand-culture with young apple trees, using ^{45}Ca as a tracer, aim at throwing some light on Ca translocation and distribution in plants under different nutrient conditions.

Materials and methods

Young apple trees (cv. Egremont Russet) grown in sand-culture with different nutrient treatments (shown below, the same as in the previous apple experiment reported by Chiu and Bould, 1977 [4]) were used for these tracer experiments.

Nutrient treatments :

1. Ca^1K^1 (*Control*) -Long Ashton standard nutrient solution, 8 meq. Ca/l, 4 meq. K/l.
2. Ca^1K^3-3 times the concentration of K in the control, other nutrients normal.
3. $Ca^{1/20}K^1$-1/20th the concentration of Ca in the control , other nutrients normal.
4. $Ca^{1/20}K^3$-1/20th and 3 times the concentrations of Ca and K in the control respective -ly, other nutrients normal.

Calcium-45 application : Four trees, one from each of the 4 treatments were treated with ^{45}Ca on 27th July, 1974 and another 4 trees were similarly treated with ^{45}Ca on 24th September, 1974 applying 70 μCi of ^{45}Ca as $^{45}CaCl_2$ on each occasion to the sand of each pot.

Fractionation of ^{45}Ca in apple leaves : Apple leaves (including old and young leaves with petioles) were taken randomly from ^{45}Ca-treated trees, cut finely into 1-2 mm pieces, weighed (about 5 g per sample) and put into plastic centrifuge tubes containing 25 ml of salt solutions (0.2M-$BaCl_2$ or-$CaCl_2$, or-$MgCl_2$, or-KCl). The mixture was shaken for 1 hour and then centrifuged (6000 RPM) for 10 minutes. The extracts were dried, ashed, dissolved in HCl and measured for ^{45}Ca activity in order to compare ^{45}Ca extractability by different cations. The ^{45}Ca of these fraction is considered as *exchang -eable* ^{45}Ca. The remaining residue, after extraction as above, was assayed for ^{45}Ca activity ; the ^{45}Ca of this fraction is considered as *fixed* ^{45}Ca.

The ^{45}Ca-treated trees were harvested on 24th August and 8th October, i. e. 28 days and 24 days after ^{45}Ca application respectively. Radioautographs and ^{45}Ca activity measurements of plant samples were made as described previously by Chiu and Bould, 1976 [2] .

Calcium-45 absorption by excised apple shoots was also investigated. The proximal ends of the cut shoots were immersed in a standard nutrient-solution (100 ml) to which ^{45}Ca (10 μCl) was added. After 24 hour absorption the shoots were prepared for radioautography.

Results

It can be seen from activity measurements (Table 1) and radioautographs (Figures

1 and 2) of mature apple leaves and roots taken from different treatments that some ^{45}Ca reached all mature leaves of normal-Ca trees, and strongly accumulated in some, 14 days after ^{45}Ca application. High-K or low-Ca treatment, or both, reduced translocation of root-applied ^{45}Ca to leaves, e. g., ^{45}Ca was not detected (by radioautographs) in some leaves from these high-K or low-Ca treatments even 14 days after^{45} Ca application. Radioautographs of roots (Figure 2) indicated that Ca-deficient roots accumulated more ^{45}Ca than Ca-sufficient roots.

Table 1. Calcium-45 activities (counts per second per g dry matter) in apple leaves and roots from trees receiviing different treatments.

Plant part (Time[a]) Treatment	1. Ca^1K^1	2. Ca^1K^3	3. $Ca^{1/20}K^1$	4. $Ca^{1/20}K^3$
Leaves (7 days)	143	24	28	38
Leaves (14 days)	300	86	163	30
Leaves (28 days)	410	124	199	143
Roots (28 days)	562	576	2099	1718

a. Time, days after^{45} Ca application.

Radioautographs (Figure 3) of the whole truss (including fruit and leaves) were made 19 days after ^{45}Ca application. Little, if any, ^{45}Ca was detected in the fruits. There was a slight accumulation of ^{45}Ca in fruit pedicels and a strong ^{45}Ca accumulation in truss leaves. Some ^{45}Ca activity in the fruits was found with other treatments, namely 145 counts per minute per g dry matter (c/m/g D. M.), on average, when ^{45}Ca was applied in July and only 7 c/m/g D. M. when it was applied in September. Results showed that little Ca moves into the fruit at the later maturing stage.

A radioautograph (Figure 4) of a ^{45}Ca-treated intact growing shoot (taken 20 days after ^{45}Ca application) showed that apical young leaves accumulated more ^{45}Ca than did basal old leaves.

Calcium-45 was quickly transported from the cut ends of excised shoots to all its leaves through the transpiration stream. Clear and strong accumulation of^{45} Ca in leaf petioles and veins can be seen in the radioautograph (Figure 5) which is very different in appearance from the radioautograph (Figure 4) of a shoot receiving root-applied ^{45}Ca which accumulated mostly in young leaves. Calcium-45 uptake and translocantoin in excised shoots would appear therefore to be through the xylem (the Ca fixing sites of which were presatureted with non-labelled Ca from the nutrient solution) to all leaves of the shoot.

Two stem lengths (each 20 cm long, taken 20-40 cm and 60-80 cm from the base) from each treatment (tree) were used for a perfusion experiment. A rubber tube was tightly connected to one end of stem into which 15 ml H_2O was poured. The perfusing solution (perfusate) was collected from the other end of the stem for measuring ^{45}Ca

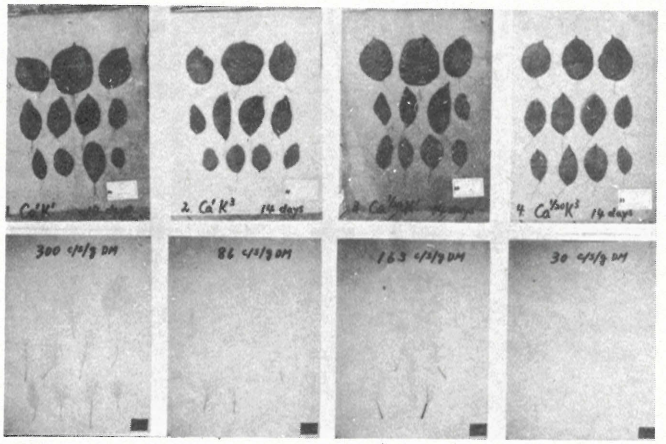

Fig. 1. Mature apple leaves (above) taken 14 days after ^{45}Ca application and their radioautographs (below). Treatments from left, (1)Ca^1K^1; (2) Ca^1K^3; (3) $Ca^{1/20}K^1$; (4) $Ca^{1/20}K^3$. Note Strong ^{45}Ca accumulation in the normal-Ca leaves and weak ^{45}Ca accumulation in the low-Ca and high-K leaves.

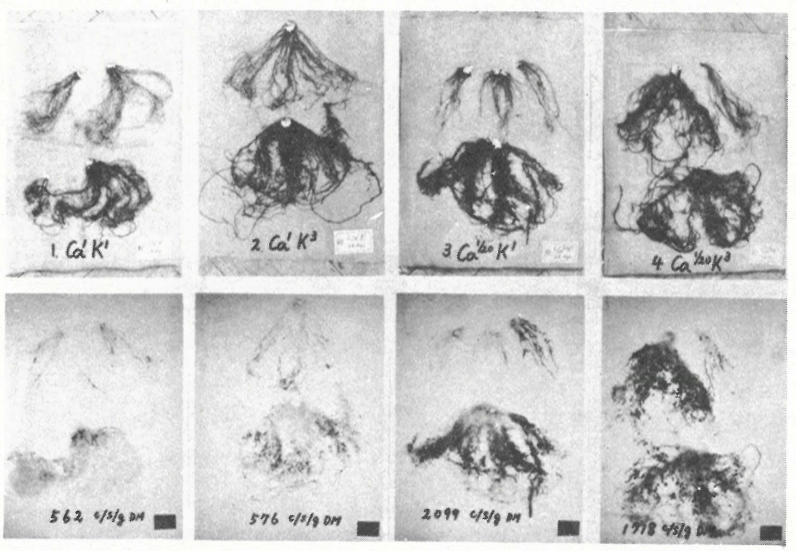

Fig. 2. Apple roots (above) taken 28 days after ^{45}Ca application. and their radic-autographs (below). Treatments from left, (1)Ca^1K^1; (2)Ca^1K^3; (3)$Ca^{1/20}K^1$; (4)$Ca^{1/20}K^3$. Note strong ^{45}Ca accumulation in the low-Ca roots and weak ^{45}Ca accumulation in the normal- Ca roots.

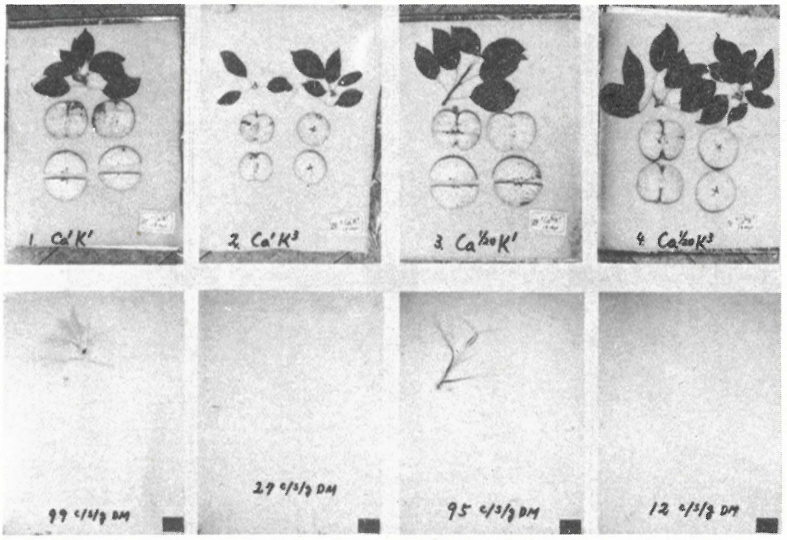

Fig. 3. Whole apple truss including fruit and leaves (above) taken 19 days after ^{45}Ca application and their radioautographs (below). Treatments from left, (1)Ca^1K^1; (2)Ca^1K^3; (3)$Ca^{1/20}K^1$; (4)$Ca^{1/20}K^3$. Note ^{45}Ca accumulated little in fruits, slightly in fruit pedicels and strongly in truss stem.

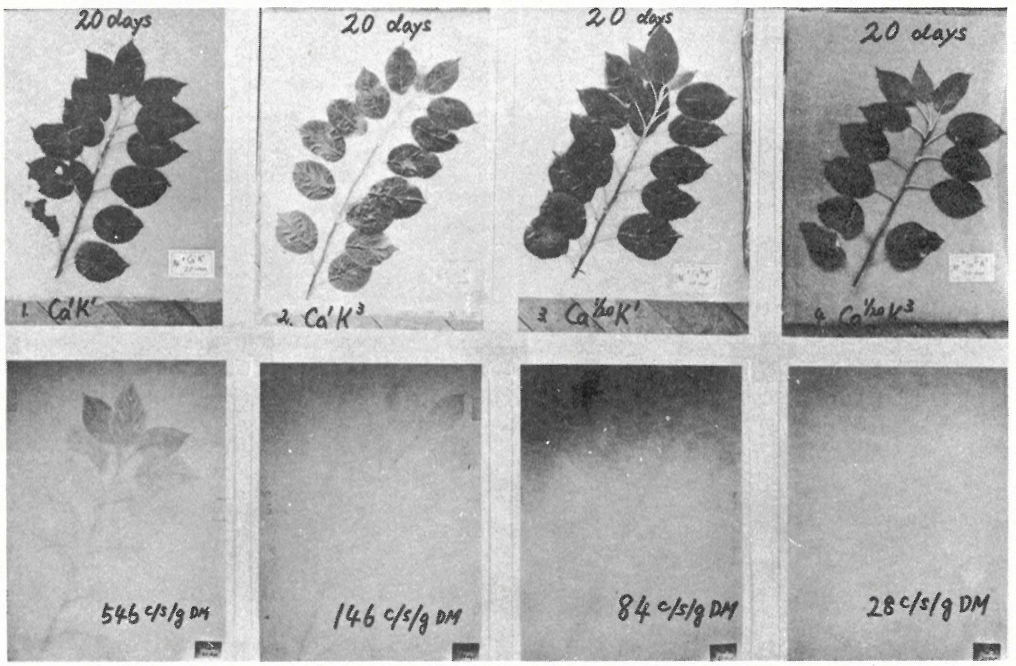

Fig. 4. Apple shoots (above) taken 20 days after ^{45}Ca application to the rocts and their radioautographs (below). Trreatments from left, (1)Ca^1K^1; (2) Ca^1K^3; (3) $Ca^{1/20}K^1$; (4) $Ca^{1/20}$ K^3. Note ^{45}Ca accumulated more in apical young leaves than in basal mature leaves. High-K and low-Ca treatments reduced translocation of root-applied ^{45}Ca to shoots.

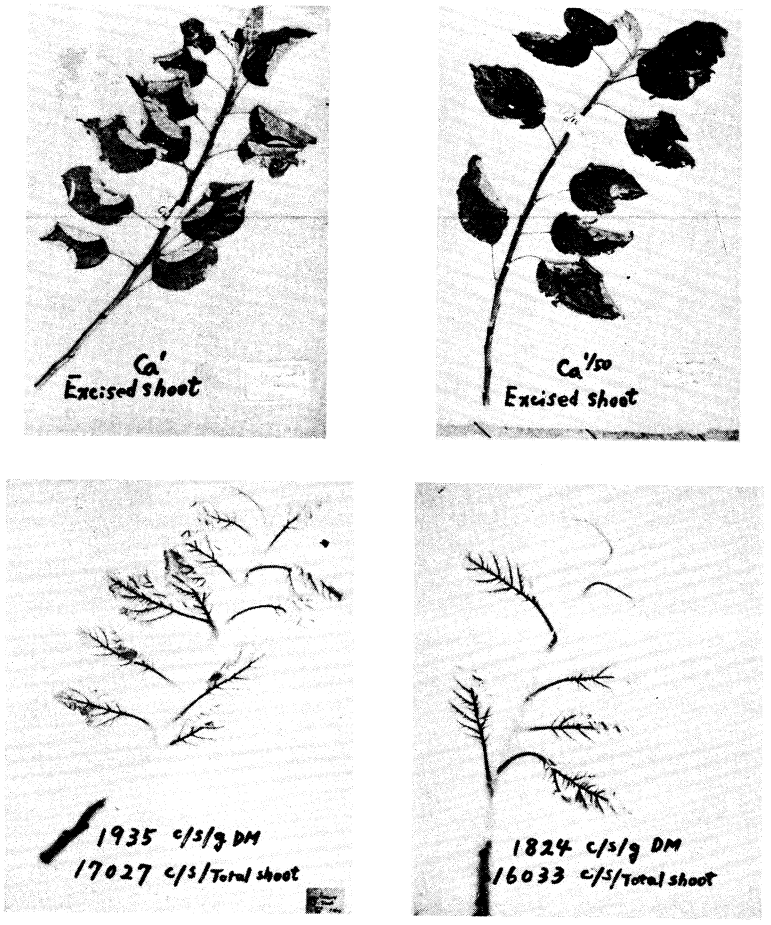

Fig. 5. Calcium-45 translocation in excised apple shoots which absorbed ^{45}Ca from the proximal ends of the cut shoots. Shoot samples(top) and their radioautographs(below). Note no difference in ^{45}Ca distribution between normal-Ca shoot (left) and low-Ca shoot (right).

activity (Figure 6). In the same manner, 15 ml of 0.5M-$CaCl_2$ and then 15 ml of N-HCl were subsequently perfused through the same stem and the perfusates were collected for measuring ^{45}Ca activities. Generally, low-Ca stems had higher total ^{45}Ca activity in perfusates than normal-Ca stem (because the Ca fixing sites in normal-Ca stems were initially more saturated with non-labelled Ca from the nutrient solutions than in low-Ca stems and fixed less ^{45}Ca). Basal stems generally had higher total ^{45}Ca activity in perfusates than apical stems (Figure 7). A similar trend was also observed in a second perfusion experiment using stems from ^{45}Ca applied in September. Decreasing gradients in ^{45}Ca activities from the base to apex of stems and to apple leaves were observed.

Comparing the percentage ^{45}Ca activities in respective perfusion solutions from different stem positions (Figure 7) , it was found that the basal stems had higher HCl-perfusate ^{45}Ca activity (54%) than $CaCl_2$-perfusate ^{45}Ca activity (45%) whereas the apical stems had higher $CaCl_2$-perfusate ^{45}Ca activity (71%) than HCl-perfusate ^{45}Ca

activity (28%). The HCl-perfusate ^{45}Ca is regarded as a fixed form of ^{45}Ca which is irreversibly bound by plant tissues. The fixed Ca constitutes most of the total Ca content of plant tissues and generally cannot be retranslocated or reutilized. Continuous increase of leaf-Ca with leaf age also suggests that there is a continuous increase in the Ca fixing sites developing in plant tissues. Calcium is adsorbed to a greater extent, and is kept for a longer time, in the basal stems than in the apical stems, therefore the former has a higher HCl-perfusate (fixed) Ca than the latter (Figure 7).

Leaf samples (taken 20 days after ^{45}Ca application in September) from each of 4 treatments were further divided into 4 samples (about 5 g each) one each of which was extracted with 25 ml of 0.2 M-$BaCl_2$, -$CaCl_2$, -$MgCl_2$ and -KCl solutions respectively. A total of 16 samples (4 treatments with 4 extracting solutions) were assayed for solution-extractable ^{45}Ca and insoluble residue- ^{45}Ca (considered as exchangeable-and fixed- ^{45}Ca respectively). Average values of Ca activity (c/m/g Fr. Wt.) for each extracting solution and treatment are shown in Figure 8. The amount of ^{45}Ca displaced by extracting solutions from leaves varied with different cations. Calcium displaced the largest amount of ^{45}Ca from leaves ; Mg was the next, then came Ba, and finally K displaced the least. Leaves from normal-Ca treatments had a higher ^{45}Ca activity than leaves from low-Ca treatments (because ^{45}Ca was more readily translocated to normal-Ca leaves). Normal-Ca leaves had the highest percentage of fixed-^{45}Ca.

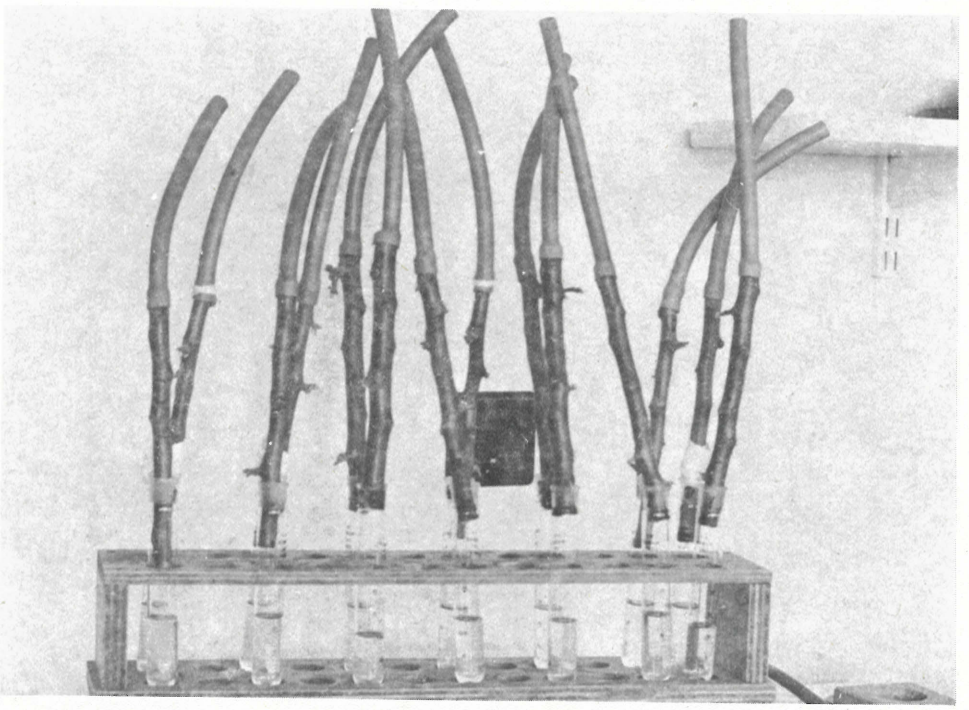

Fig. 6. Perfusion experiment of ^{45}Ca apple stems. A solution, water or salt, was poured into a rubber tube which was tightly connected to one end of the stem. Perfusion solution was collected in glass tube from the otherend of the stem for measuring ^{45}Ca activity .

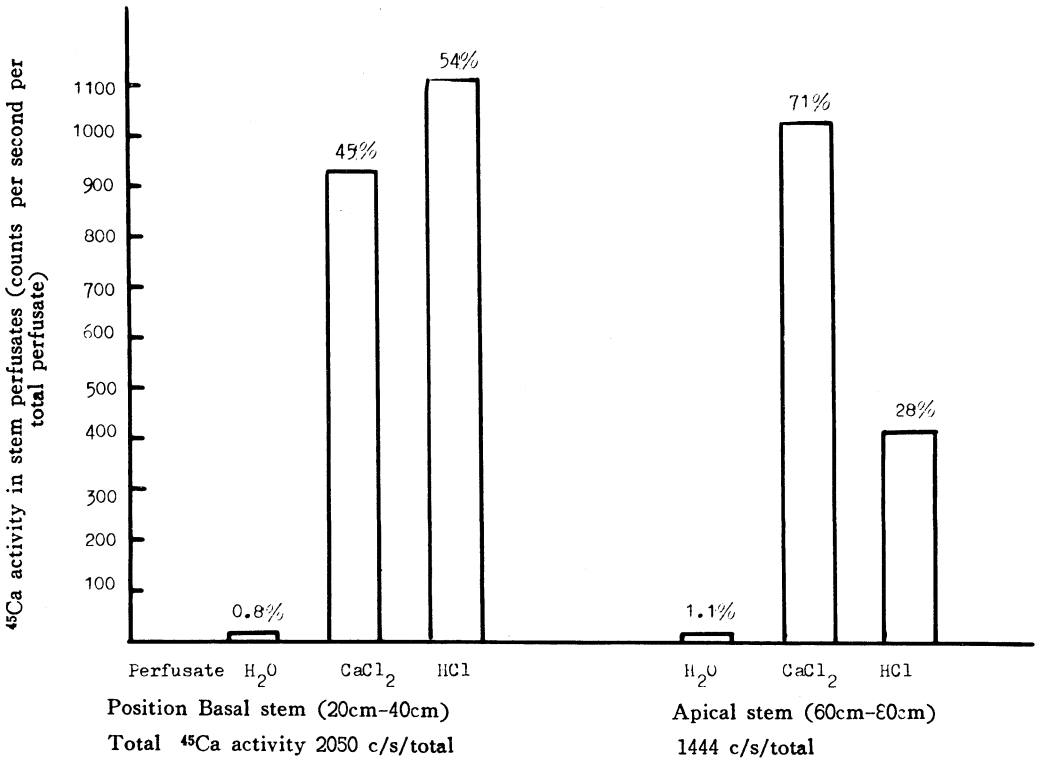

Fig. 7. Calcium-45 activities, and their percentage activities, in perfusates from different position of apple stems perfused with H_2O, 0.5M-$CaCl_2$ and N-HCl solutions. (Stem samples taken 28 days after ^{45}Ca root application: the data show values of 7 samples.)

Discussion

The characteristics of ^{45}Ca mobility in plant tissues appear to be similar in different plant species, as demonstrated by current experiments on apple trees, strawberry [2] and tomato plants [3]. High-K level decreased ^{45}Ca translocation in all palnts. Calcium-45 accumulated mainly in the base (roots, stem of crown) of Ca-deficient plants and was readily translocated from roots to tops of Ca-sufficient plants. Root-absorbed ^{45}Ca accumulated much more in basal leaves than in apical leaves of tomato plants. However, in attached apple shoots, irrespective of normal or low-Ca pre-treatment, ^{45}Ca accumulated more in apical young leaves than in basal old leaves (Figure 4) indicating that Ca transport is not controlled entirely by the transpiration stream. The difference in ^{45}Ca translocation between apple shoots and tomato plants may be due to the differente (internal regulatory) character of woody stems (apple shoot) and herbaceous stems (tomato plant). Millikan [9] reported that root-absorbed ^{45}Ca moves preferentially into the laminae of younger, rather than into the older, leaves of the terminal shoots of apple trees. Wieneke and Fuhr [13] also found that more ^{45}Ca was transported into rapidly-developing leaves than into old leaves of apple trees. It has also been reported that plants have hormonal means of regulating their Ca mobility and distribution [1,6,12].

This characteristic of[45] Ca mobility in apple shoots explains why leaf marginal scorch symptoms (caused by Ca stress) appear in the basal old leaves first and not in the apical young leaves, as reported in our previous paper [4]. The Ca fixing sites in leaf tissues, which need to be saturated with Ca before re-export can take place, are continuously developing during leaf growth. When Ca is irreversibly fixed by these sites (constituting most of the leaf total Ca), this fixed Ca is no longer available for functional use. Therefore a continuous supply of newly-absorbed Ca is required during leaf growth and maturation. In Ca-deficient apple shoots, the limited supply of root-absorbed Ca is mostly translocated to the younger leaves, therefore older leaves are deprived of functional Ca and develop leaf marginal scorch (a Ca deficiency symptom-in serious cases, young leaves and the tops of shoots also show scorch symptoms). It appears that a hormonal regulatory system might also be involved in Ca translocation within plants. Generally, the most sensitive plant tissue to Ca deficiency is in that plant organ into which Ca is most difficult to move, e. g. apple (bitter pit), tomatoes (blossom-end rot), the tops of plants (die-back of tomato plants ; leaf tipburn of strawberry).

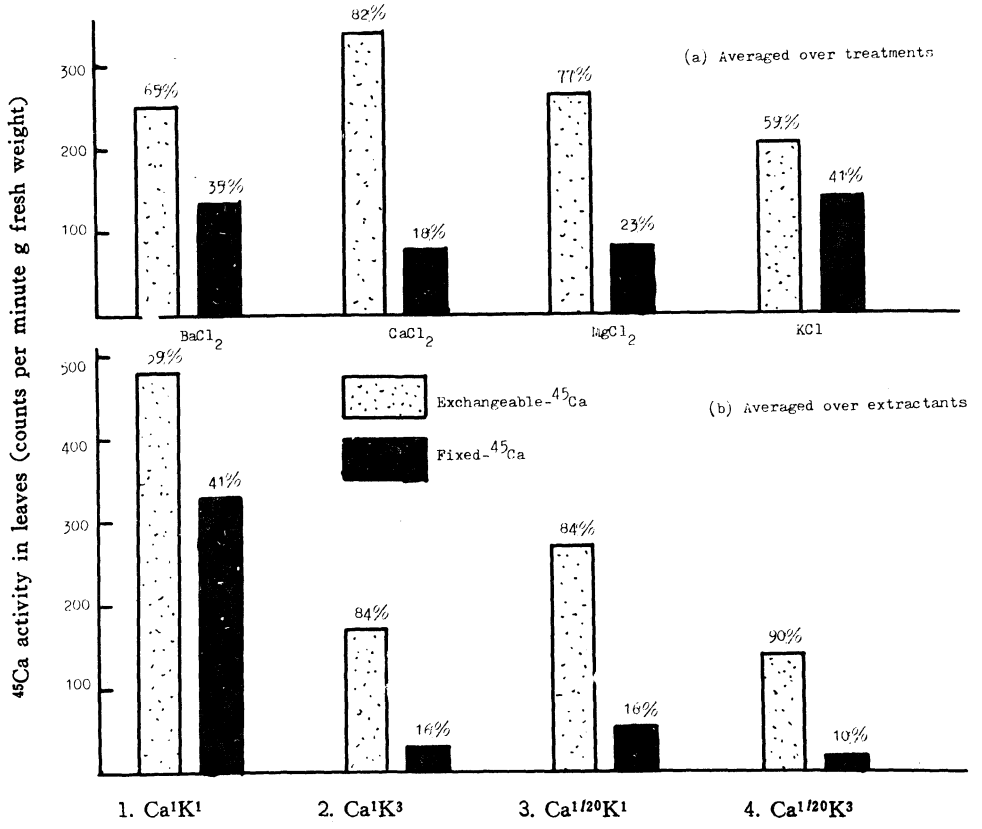

Fig. 8. Exchangeable-and fixed-[45] Ca activities (c/m/g Fr. Wt.) and their percentage activities in apple leaves (from different nutrient treatments) extracted with 0.2M-$BaCl_2$, -$CaCl_2$, -$MgCl_2$ and -KCl solutions. (Samples taken 20 days after[45] Ca application: the data show mean values of 4 samples.)

The plant-tissues capacity for Ca fixation (i. e. the amount of ^{45}Ca accumulated per unit plant weight) varies according to the general nutritional status of the plant. For instance, Ca-deficient roots accumulated much more ^{45}Ca than did Ca-sufficient roots (Table 1). Therefore, the pre-nutritional status of plants changes the mobility of applied Ca through its effect on the Ca-fixing sites in the plant tissues. The Ca fixing sites are not entirely non-selective because the amount of ^{45}Ca that can be replaced by concentrated solutions of other divalent cations (0.2M-$BaCl_2$ or-$MgCl_2$) from previously-deposited ^{45}Ca in leaves, is much less than that displaced by non-radioactive Ca (0.2M-$Ca(NO_3)_2$) solution (Figure 8). Root-absorbed ^{45}Ca, after reaching the fixing sites, is gradually turned into irreversibly-bound form, as shown in the greater ^{45}Ca fixation capacity of basal stems (Figure 7) and in the increase of leaf Ca content (mostly non-exchangeable fixed form) with leaf age.

From our Ca mobility studies, a hypothesis of *continuously-developing calcium-fixing sites* in growing tissues has been suggested. The Ca-fixing sites, which are not entirely non-selective, are ceaselessly requiring additional Ca in order to maintain all cellular functions and intact membrane structure. There are at least two categories of plant Ca, namely ; (1) irreversibly-bound Ca by the fixing sites (that hold the major plant total Ca, and which cannot be retranslocated) and (2) reversibly-adsorbed (exchangeable) Ca that can be displaced by other cations to some extent. This system of fixed-Ca and adsorbed-Ca, which is in dynamic equilibrium during plant growth, controls the supply of functional Ca. Old apple leaves with a high Ca content, but showing Ca deficiency symptoms (marginal scorch) support the explanation that most of the Ca in old leaves is irreversibly fixed. A limited supply of root-absorbed Ca (which is preferentially transslocated to young leaves) cannot satisfy the adidtional requirement of newly-developed Ca-fixing sites in old leaves which results in the appearance of Ca deficency symptoms. Thus a calcium deficiency may appear in old leaves in the presence of apparent (total Ca) plenty. A small continous supply of Ca is required by plants if Ca deficiency in rapidly-growing tissues is to be avoided. The best way of preventing Ca deficiency is to maintain a continous movement of Ca into sensitive tissues (and to avoid interfering factors, e. g. high-k etc.) by ample supplies of Ca through the root medium, or by direct Ca sprays to plants during the period when Ca deficiency disorders are most likely to arise.

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在不同鈣營養條件下蘋果幼樹之鈣-45運移[1]

邱 再 發[2]

摘 要

施用於根部之鈣-45在正常鈣營養之果樹比在高濃度鉀或缺乏鈣營養之果樹較容易運移至葉部。後者果樹比前者果樹鈣-45聚積更多於其根部及莖基部，施用於根部之鈣-45在生長中之枝條運移至上端新葉比至基部老葉爲多。施用於切斷枝條之斷端之鈣-45迅速的經過導管之蒸散流運移至枝條上所有的葉片。在果實生長後期，果樹所吸收之鈣-45頗難進入果實內，而聚積於果實附近之葉片。一般果樹之無機營養狀態可影響其組織中之鈣吸附及固定能力，而改變鈣在植物體之移動性，生長中之植物組織有繼續產生固定鈣的基質。

1. 臺灣省農業試驗所 研究報告 第 935 號。
2. 本所農業化學系技正。臺灣省 臺中縣 霧峰鄉。